Confirmation and Efficacy Tests Against Codling Moth and Oriental Fruit Moth in Apples Using Combination Heat and Controlled Atmosphere Treatments

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ABSTRACT Codling moth, *Cydia pomonella* (L.), and oriental fruit moth, *Grapholita molesta* (Busck), are serious pests of apples (*Malus* spp.) grown in the United States and other countries. In countries where these species are not found, there are strict quarantine restrictions in place to prevent their accidental introduction. The treatment used in this study consisted of hot, forced, moist air with a linear heating rate of 12°C/h to a final chamber temperature of 46°C under a 1% oxygen and 15% carbon dioxide environment. We found that the fourth instar of both species was the most tolerant to the treatment, with equal tolerance between the species. Efficacy tests against the fourth instar of both oriental fruit moth and codling moth by using a commercial controlled atmosphere temperature treatment system chamber resulted in >5,000 individuals of each species being controlled using the combination treatment. Confirmation tests against codling moth resulted in mortality of >30,000 fourth instars. These treatments may be used to meet quarantine restrictions for organic apples where fumigation with methyl bromide is not desirable.

KEY WORDS codling moth, oriental fruit moth, apple, quarantine, CATTS

The presence or potential presence of codling moth, *Cydia pomonella* (L.), and oriental fruit moth, *Grapholita molesta* (Busck), in apples (*Malus* spp.) grown in United States has caused the development and implementation of specific quarantine procedures to prevent the accidental introduction of these pests into areas where they do not occur (codling moth in Japan; oriental fruit moth in Mexico and British Columbia, Canada). Mexico, Taiwan and British Columbia require the issuance of a phytosanitary certificate under a systems approach (NWHC 2006). Although organic apples can meet the stringent provisions of the systems approach to meet quarantine requirements, the requirement for methyl bromide fumigation for entry into Japan does not coincide with current U.S. and Japan organic standards (McEvoy 2006, NOP 2006).

Most nonchemical quarantine treatments involve the application of extreme high or low temperatures (Neven 2000, 2003; Wang et al. 2006). Combinations of low temperature and controlled environments (CAs) (low oxygen, elevated carbon dioxide) also have been widely used (Hallman 1994). High temperature treatments have been traditionally developed for tropical and subtropical fruits and vegetables (Armstrong 1994). Low temperature CA treatments were seen as the only viable method of disinfestation for temperate crops, such as apples and pears. However, research from Israel indicated that apples could handle high temperature treatments and that these treatments could alleviate some postharvest disorders such as superficial storage scald (Klein et al. 1990; Lurie et al. 1990, 1991; Klein and Lurie 1992; Klein 1994). Although the temperatures and heating rates used in these studies did not effectively control internal feeding pests such as codling moth (Neven and Rehfield 1995), the results did point the way for the development of heat treatments for apples to control these pests (Neven et al. 1996; Neven 1998a,b).

Plants have a relatively high capacity for anaerobic metabolism, whereas insects have a very limited capacity for anaerobic metabolism. The presence of oxygen is critical for insect acclimation to thermal stress (Yocum and Denlinger 1994, Neven 2003). In addition, terrestrial insect respiration is chiefly regulated by the presence of carbon dioxide. These differences in plant and insect physiological responses to thermal and respiratory stress were capitalized on by the invention of the controlled atmosphere temperature treatment system (CATTS) technology (Neven and Mitcham 1996). This system combined the application of moist or vapor forced hot air under a controlled atmosphere. This technology is similar to existing vapor and forced air treatment systems currently approved and in use for several commodities entering countries worldwide. The only difference in CATTS is the application of a controlled atmosphere. However, this addition is critical for the success of this treatment in providing appropriate pest control and for preserv-

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ing commodity quality. Research using CATTS or CATTS-type technology has been increasing through the past 10 yr (Neven and Mitcham 1996; Whiting and Hoy 1997; Whiting et al. 1999; Neven and Drake 2000a,b; Yahia 2000a,b,c; Neven et al. 2001; Shellie et al. 2001; Neven 2004, 2005; Obenland et al. 2005).

This report describes a body of work encompassing >9 yr of research on the development of a CATTS treatment for disinfestation of apples from codling moth and oriental fruit moth. We performed tests to determine the most tolerant stage to CATTS treatments, the most tolerant species of the most tolerant stage, efficacy tests of both codling moth and oriental fruit moth in a commercial CATTS treatment unit, and confirmation tests against codling moth in the research CATTS unit. Efficacy tests were conducted in the commercial CATTS unit to demonstrate commercial viability of the technology and treatment. Confirmation tests were performed in the research CATTS unit for reproducibility of such a large number of tests needed to treat >30,000 insects in apples.

Materials and Methods

Insects. Both codling moth and oriental fruit moth were reared in the laboratory by using the same wheat germ-based artificial diet originally developed for codling moth (Toba and Howell 1991). Insects were reared at 23 ± 2°C, 50% RH, and a photoperiod of 16:8 (L:D) h. First instars were collected from wax-coated oviposition bags (9 by 15 by 27.5 cm; depth by width by length) containing 250 mating pairs (500 total). Females were allowed to oviposit for 24–48 h at 23 ± 2°C, 50% RH, and a photoperiod of 16:8 (L:D) h. The bag was placed at 2°C for 5 min to facilitate removal of moths. The bags were held at 23 ± 2°C, 50% RH, and a photoperiod of 16:8 (L:D) h until the desired egg stage was achieved or first instars emerged. For codling moth, white ring (WR) was 0–48 h, red ring (RR) was 48–72 h, and blackhead (BH) was 96–120 h. Larvae from the second through fifth instar for codling moth and second–fourth instar for oriental fruit moth were removed from the artificial diet by hand and placed directly onto the fruit to be used for testing. Apples were held at 23 ± 2°C, 50% RH, and a photoperiod of 16:8 (L:D) h for 20 h to allow for insects to enter the fruit. Tests with eggs were performed by placing 250 2-d-old moths onto 4.54 kg of organic apples that were placed into a plastic box (8.5 by 27 by 37 cm); the bottom and top were lined with unbleached muslin to prevent oviposition on the plastic box. Fruit with moths were held for 24 h at 23 ± 2°C, 50% RH, and a photoperiod of 16:8 (L:D) h after which the box was placed at 2°C for 5 min to facilitate removal of moths. The boxes containing fruit with eggs on them were held at rearing conditions until the desired egg stage was reached.

Organic apples, normally 'Golden Delicious', but sometimes 'Red Delicious' or 'Gala' were used as hosts for infestation with codling moth and oriental fruit moth larvae. The fruit were divided into 6.82-kg lots in plastic storage boxes (41 by 57.5 by 13.2 cm). In total, 200 larvae were applied to the 6.82 kg of mature fruit. The top of the box was lined with double stick tape to which a length of nylon organdy was adhered to prevent larvae from exiting the box. The top was then sealed with the lid to the storage box. The infested fruit was held at 23 ± 2°C, 50% RH, and a photoperiod of 16:8 (L:D) h overnight. A group of 100 larvae of the same stage were used to infest 3.41 kg of fruit to serve as untreated controls. Before treatment, infested fruit was removed from the boxes and transferred into the CATTS treatment lugs (OnoPac, Hilo, HI). Any insects outside of the fruit were counted and subtracted from the total infested number to obtain the total number of insects actually receiving the treatment. After treatment, fruit was stored at 0°C overnight to prevent accidental reinfestation. Previous research (Neven 1994, Neven and Rehfield 1995) showed minimal effects of overnight cold storage on mortality. Controls also were stored at 0°C overnight and were used for calculations for corrected mortality. After removal from cold storage, fruit were allowed to warm up to room temperature and examined for live, dead or moribund larvae or egg hatch. Moribund larvae were placed onto organic apples and placed under normal rearing conditions for 7 d, after which the fruit was examined for live or dead larvae. Fruit with eggs on them were held an additional 7–10 d to allow for egg hatch, depending on the stage treated.

CATTS Treatments. The treatment consisted of a 12°C/h linear chamber heating rate up to a final chamber temperature of 46°C, under a 1% O_2, 15% CO_2, >90% RH atmosphere with air speed between 1.3 and 2 m/s for a duration of 3 h (total treatment time). Core temperature of the fruit reached 44.6°C and remain there for 15 min (Fig. 1). After treatment, fruit were removed from the chamber and forced air cooled in a 0°C cold room. Fruit were examined for hatched eggs or surviving larvae as described above.

Egg and Larval Tolerance to CATTS Treatments. The three egg stages and all the larval stages of both codling moth and oriental fruit moth were tested for their relative tolerance to CATTS treatments of apples. For egg-stage tolerance, groups of 250 2-d-old moths were exposed to 4.54 kg of organic Red Delicious apples for a 24-h period as described previously. The containers were chilled for 5 min, and the moths were aspirated off with vacuum. Apples with eggs on them were kept in an environmentally controlled room at 23 ± 2°C, 50% RH, and a photoperiod of 16:8 (L:D) h until the desired stage was attained. On the day of treatment, the eggs were examined for stage and counted. At least 120 eggs per time point per replication were used. First and second instars were placed onto the apple at a rate of 12 larvae per fruit. Third through fourth (oriental fruit moth) or fifth (codling moth) instars were placed onto the apple at a rate of six per fruit. Each treatment (time × replicate) contained 120 larvae. The five time points used were 0, 1.5, 2, 2.5, and 3 h. The zero time point was used as the untreated control. Each time point was replicated four times. The CATTS treatment of 12°C/h, final chamber temperature of 46°C under a 1% O_2, 15% CO_2, −2°C

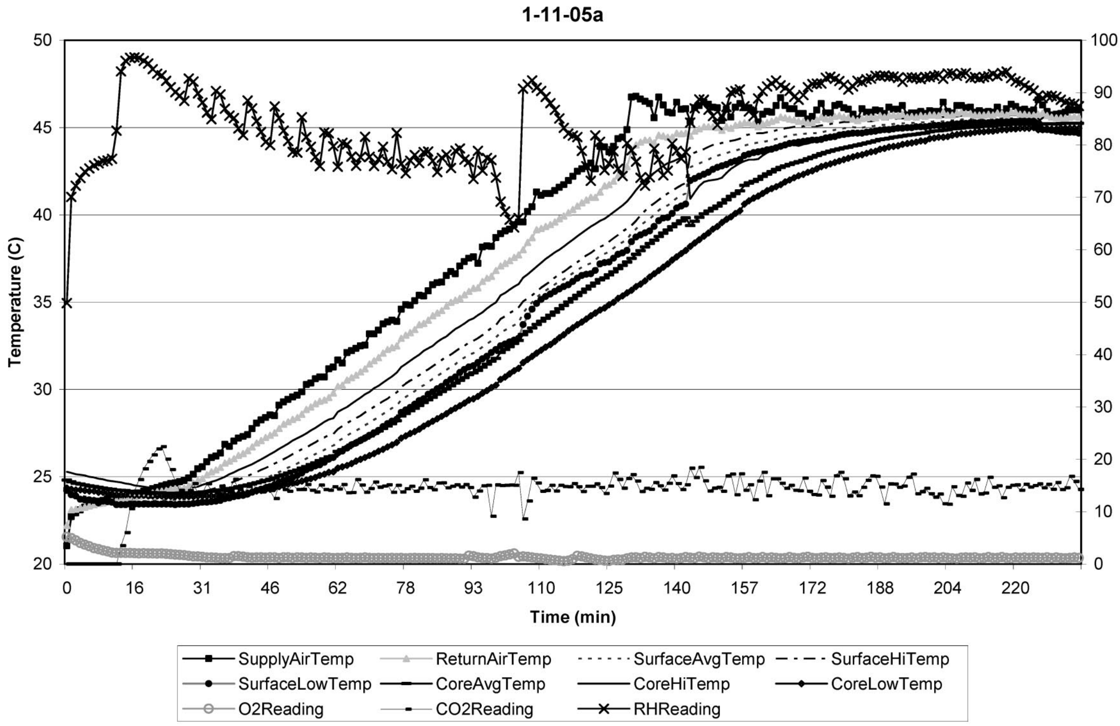

Fig. 1. Treatment conditions and fruit temperatures during a typical CATTS efficacy treatment in the commercial CATTS chamber. Relative humidity, oxygen, and carbon dioxide levels indicated by the secondary 'atmospheres' axis.

dew point environment and 1.2 m/s air speed was used for these treatments.

Species Tolerance to CATTS Treatments. Most tolerant species treatments were performed as described for the determination of the most tolerant life stage with the exception that only fourth instars of codling moth and oriental fruit moth were used, and CATTS treatments were performed under air and CA environments (1% O_2, 15% CO_2) (see Results). Time points consisted of 0, 1.5, 2, 2.5, and 3 h. Treatments and evaluations were performed as previously described. The 0-h time point was used as the control from which control mortality was calculated and used in Abbott's corrected mortality calculation (Abbott 1925) for determination of corrected mortality of treatments.

Efficacy Tests. Efficacy tests were performed in the 2-ton commercial CATTS chamber located at PacOrganic in George, WA, during spring 2004. The commercial CATTS was designed by Techni-Systems of Chelan, WA, and holds four standard apple bins weighing 1,000 lb (454.5 kg) each. The unit operates under a 480-V, three-phase power, 47-amp power supply. There are eight heaters providing 21,600 W of heat. Along with the heaters there are eight 1.5 horsepower fans and eight fogging misters capable of supplying 0.5 gal/h humidification water to the unit. The dimensions are 367.5 by 272.5 by 167.5 cm (height by width by depth). The center section of the back wall is removable for addition of more chambers. The unit has 16 temperature probes (10-k ohm thermistors, Thermometrics New Jersey, Edison, NJ) and an oxygen and carbon dioxide analyzers (TS-1100, Techni-Systems made for Pacific California, Yakima, WA) for monitoring the atmospheres. The unit also contains a relative humidity transmitter (model HO1NMSX, Veris Industries, Portland, OR) for measuring humidity.

Organic apples were infested with early fourth instars of codling moths or oriental fruit moths. There were 200 larvae applied to 6.82 kg of organic apples. The apples and larvae were held overnight at 23 ± 2°C to allow the larvae to enter the fruit. The next day, the fruit containing the larvae were placed into a 105.6- by 105.6- by 60-cm nylon organdy bag inside a plastic apple bin (Macro Plastics, Yakima, WA). All larvae on the outside of the fruit were removed and counted. The number of larvae found outside of the fruit was subtracted from the infested total and the remaining number was used as the total number of insects actually receiving the treatment. After placement of the infested fruit into the nylon organdy bag, the bag was sealed with duct tape and paper clamps. Filler fruit was used to occupy the remaining space in the apple bin to provide a full bin for treatment.

The bin was then transported to PacOrganic. Control fruit was included in the load. The infested fruit was placed in the lowest position in the commercial CATTS chamber, because previous thermal mapping determined this location to be the cold spot. In total,

Table 1. Effect of CATTS treatments on egg hatch and mortality of the different larval stages oriental fruit moth

Stage	LT_{50}	Lower 95% CL	Upper 95% CL	LT_{90}	Lower 95% CL	Upper 95% CL	LT_{99}	Lower 95% CL	Upper 95% CL	df	Intercept χ^2	Time χ^2	Intercept Pr > χ^2	Time Pr > χ^2
1	2.14	1.95	2.32	2.72	2.52	3.03	3.12	2.86	3.55	13	6.12	6.45	<0.0001	<0.0001
2	2.18	1.99	2.37	2.77	2.57	3.09	3.17	2.91	3.61	13	6.33	6.54	<0.0001	<0.0001
3	2.31	2.11	2.51	2.84	2.63	3.19	3.21	2.94	3.70	13	5.64	5.74	<0.0001	<0.0001
4	2.36	2.23	2.46	2.71	2.56	2.96	2.96	2.77	3.32	18	5.60	5.55	<0.0001	<0.0001
WR	1.93	1.19	2.58	2.60	2.19	4.15	3.04	2.54	5.19	8	2.76	3.07	0.0058	0.0021
RR	1.69	1.21	2.07	2.31	1.99	3.08	2.70	2.32	3.79	8	3.21	3.82	0.0013	0.001
BH	1.62	1.19	1.96	2.22	1.94	2.88	2.61	2.26	3.54	8	3.37	4.07	0.0008	0.0001

Lethal times are listed in hours with 95% CL.

15 temperature probes were placed in the fruit in the bag and in the filler fruit. Fruit core temperatures were monitored at 10 locations in the bag and five locations in filler fruit above the bag.

Treatment conditions consisted of a 15-min hold at 23°C to establish atmospheres of 1% O_2, 15% CO_2, −2°C dew point. Then, the ramped heat treatment of 12°C/h to a final chamber temperature of 46°C was applied. Tests were ended when the core average temperature had maintained a minimum of 44.5°C for 15 min. After treatment, the bin containing the infested fruit was transported back to Yakima Agricultural Research Laboratory (Wapato, WA) and stored at 0°C until evaluation the next day. Short-term cold storage has minimal effect on fourth instar mortality (Neven 1994). Cold storage was used to prevent accidental reinfestation of the treated fruit. Fruit were cut open to determine live, dead, or moribund larvae. Moribund larvae were placed on organic apples and held under normal rearing conditions for 7 d, after which the fruit was cut open to determine the fate of the larvae.

Confirmation Tests. Organic apples were infested with early fourth instars of codling moths and handled as described in the efficacy tests, with the exception that the confirmation tests were conducted in the laboratory CATTS chamber at the Yakima Agricultural Research Laboratory. Treatment consisted of a 15-min hold at 23°C to establish an atmosphere of 1% O_2, 15% CO_2, −2°C dew point (Fig. 1). Then the ramped heat treatment of 12°C/h to a final chamber temperature of 46°C was applied. Tests were ended when the lowest core temperature had maintained a minimum of 44.5°C for 15 min. After treatment, lugs containing the infested fruit were stored at 0°C until evaluation the next day. Fruit were cut open to determine live, dead, or moribund larvae. No moribund larvae were found in these tests.

Statistics. Control mortality was calculated using Abbott's formula (Abbott 1925). Data were tabulated in Excel 2000 (Microsoft, Redmond, WA) where initial calculations on control mortality and standard error were performed. Probit analysis was performed using PROCPROBIT in SAS version 8.2 (SAS Institute 2000). Time was squared, and mortality was not converted. Factorial analysis of variance (ANOVA) was performed on mortality data by using SAS where mortality was converted to the arcsine of the square root. Means were separated using Duncan's new multiple range test. Analysis of fruit core temperatures was performed on QuattroPro 10 (Corel Inc., Dallas, TX) where averages, minimum, and standard error of the mean were calculated from treatment temperature data obtained from CATTS treatments. Differences between core temperatures were determined using F-test in QuattroPro 10 and ANOVA by using SAS (SAS Institute 2000).

Results

Most Tolerant Life Stage. Probit analysis of the immature stages of oriental fruit moth indicate that the fourth instar is the most tolerant stage (Table 1; Fig. 2). However, overlapping confidence limits (CLs) makes definitive determination difficult. When both larvae and eggs of the oriental fruit moth were compared, factorial ANOVA indicated that the third and fourth instars were equally tolerant and were more tolerant than the other larval and egg stages and that the egg stages were equally tolerant to one another ($F_{6, 59} = 15.70$; $P < 0.0001$) based on Duncan's means separation test. When only larval stages were compared, the fourth instar was determined to be the most tolerant ($F_{3, 44} = 5.81$; $P = 0.0020$) with the second and third instars being equal and the first instar being the least tolerant based on Duncan's means separation test. There was no significant interaction between stage and time ($F_{12, 44} = 1.33$; $P = 0.2383$). When only egg stages were compared, there was no difference in the stages ($F_{2, 15} = 0.63$; $P = 0.5442$), and there was no interaction between stage and time ($F_{8, 15} = 0.71$; $P = 0.6761$).

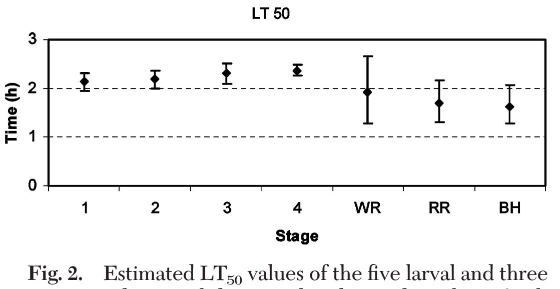

Fig. 2. Estimated LT_{50} values of the five larval and three egg stages of oriental fruit moth subjected to the 12°C/h CATTS treatment. Error bars indicate 95% CL. Instars indicated as numbers 1–4.

Table 2. Effect of CATTS treatments on mortality of the different larval stages of codling moth

Stage	LT_{50}	Lower 95% CL	Upper 95% CL	LT_{90}	Lower 95% CL	Upper 95% CL	LT_{99}	Lower 95% CL	Upper 95% CL	df	Intercept χ^2	Time χ^2	Intercept Pr > χ^2	Time Pr > χ^2
1	2.12	1.96	2.29	2.62	2.44	2.90	2.96	2.74	3.34	18	38.64	40.5	<0.0001	<0.0001
2	2.07	1.91	2.22	2.75	2.57	3.01	3.20	2.97	3.55	18	55.61	65.77	<0.0001	<0.0001
3	2.19	1.99	2.40	2.65	2.45	3.01	2.98	2.72	3.47	18	25.56	26.56	<0.0001	<0.0001
4	2.29	2.04	2.57	2.73	2.50	3.24	3.05	2.75	3.75	18	16.69	17.01	<0.0001	<0.0001
5	2.34	2.03	2.68	2.73	2.48	3.47	3.02	2.71	4.06	18	10.35	10.35	0.0013	0.0012
WR	1.99	1.59	2.23	2.71	2.40	3.45	3.86	2.78	4.24	8	13.92	17.77	0.0002	<0.0001
RR	1.99	1.59	2.34	2.72	2.36	3.64	3.23	2.77	4.54	8	10.25	14.98	0.0014	0.0001
BH	1.05	0	1.63	2.48	2.12	3.44	3.22	2.71	4.79	8	0.81*	11.6	0.3668*	0.0007

Lethal times are listed in hours with 95% CL.
Asterisk (*) indicates not significant.

Probit analysis of the immature stages of codling moth indicated that the fourth and fifth instars were the most tolerant stages and equally tolerant to one another (Table 2; Fig. 3). However, overlapping confidence limits made definitive determination difficult. Factorial ANOVA of the mortality of codling moth in apples indicated that all larval instars were equally tolerant and were more tolerant than the eggs stages, which were all equal in tolerance ($F_{7,\ 75} = 12.2$; $P < 0.0001$) based on Duncan's means separation test. There was no significant interaction between time and stage ($F_{28,\ 75} = 1.35$; $P = 0.1554$). When only larval stages were compared with one another, there was again no difference between the stages ($F_{4,\ 75} = 2.41$; $P = 0.567$), and there was no significant interaction between stage and time ($F_{16,\ 75} = 1.67$; $P = 0.0723$). When eggs were compared with one another, there was no difference between the stages ($F_{2,\ 20} = 2.95$; $P = 0.0755$), and there was no significant interaction between stage and time ($F_{8,\ 20} = 0.72$; $P = 0.6758$).

When the larval stages of codling moth and oriental fruit moth were compared using factorial ANOVA, there was a significant interaction between stage and time ($F_{16,\ 119} = 2.09$; $P = 0.0131$). When one-way ANOVAs were performed by stage, all stages were significant for time. When one-way ANOVAs were run by time, only the 2.5- and 3-h time points were not significant.

We also found that there was no difference in species tolerance ($F_{1,\ 119} = 0.15$; $P = 0.6991$) but that the fourth instar was the most tolerant stage ($F_{4,\ 119} = 5.7$; $P = 0.0003$) based on Duncan's means separation test. There was no significant interaction between stage and species ($F_{3,\ 119} = 0.65$; $P = 0.5842$), time and species ($F_{4,\ 119} = 1.65$; $P = 0.1674$), and stage × time × species ($F_{12,\ 119} = 0.65$; $P = 0.7913$).

When the egg stages of both species were compared using factorial ANOVA, there was a significant interaction between time and species ($F_{4,\ 15} = 1.63$; $P = 0.02184$). When one-way ANOVA was performed, all egg stages were significant for time, but only the 2-h time point was significant for all egg stages ($F_{2,\ 10} = 4.28$; $P = 0.0454$). There was no difference between the species ($F_{1,\ 35} = 0.01$; $P = 0.9343$) or among the stages ($F_{2,\ 35} = 1.59$; $P = 0.2369$). There was no significant interaction between stage and species ($F_{2,\ 35} = 0.39$; $P = 0.6835$), stage and time ($F_{8,\ 15} = 0.6$; $P = 0.7603$), or stage × time × species ($F_{8,\ 15} = 0.23$; $P = 0.9799$).

Most Tolerant Species. When treatments of codling moth performed under air were compared with those performed under a controlled atmosphere, we noticed a marked increase in mortality in insects treated under the controlled atmosphere (Fig. 4). At the 3-h time point, larval mortality was only 50% in the treatment performed under air, whereas mortality was 100% under the CA conditions. Treatments of both codling moth and oriental fruit moth under CATTS conditions (Fig. 5) showed that codling moth is slightly more tolerant to oriental fruit moth at the 2- and 3-h time points.

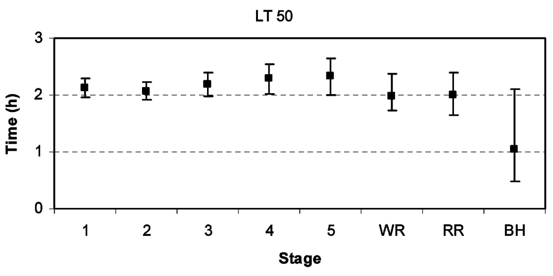

Fig. 3. Estimated LT_{50} values of the five larval and three egg stages of codling moth subjected to the 12°C/h CATTS treatment. Error bars indicate 95% CL. Instars indicated as numbers 1–5.

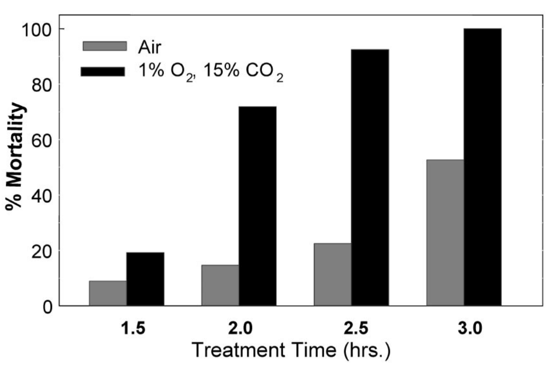

Fig. 4. Percentage of corrected mortality of fourth instar codling moth in apples by using a heating rate of 12°C/h to a final chamber temperature of 46°C under air (▨) and CA (■) conditions (1% O_2, 15% CO_2).

Table 3. Mortality of fourth instars of codling moth and oriental fruit moth to 12°C/h, 46°C, 1% O_2, 15% CO_2, −2°C dew point CATTS treatment

Species	Control no.	% control mortality	No. infested	No. treated	% mortality
Codling moth	150	1.5	6,423	5,827	100
Oriental fruit moth	200	3.2	7,200	5,497	100

Treatments were performed in a 1,000-lb commercial plastic bin in the 2-ton commercial CATTS chamber at George, WA.

Efficacy Tests. The CATTS treatment in the commercial bins of apples was sufficient to control >5,000 codling moth and >5,000 oriental fruit moth fourth instars (Table 3). In total, 6,423 fourth instars of codling moth were applied to apples, resulting in 5,827 remaining in the fruit, which were killed with the CATTS treatment. In total, 7,200 fourth instars of oriental fruit moth were applied to the apples, resulting in 5,497 remaining in the fruit, which were killed by the CATTS treatment.

Confirmation Tests. There were 39,800 fourth instars applied to the apples (Table 4) over the course of 20 separate treatment runs. There were 8,469 larvae removed from outside of the fruit before CATTS treatment. We treated 31,331 larvae in the CATTS treatment. With an estimated control mortality of 1.5%, the corrected number of treated larvae was calculated to be 30,861 with zero survivors.

Treatment Efficacy. Analysis of the average and minimum low core fruit temperatures resulting in 100% kill of codling moth and oriental fruit moth were performed to determine requirements defining treatment efficacy. We took the lowest core temperatures at the end of a treatment as well as those 15 or 30 min from the end of the treatment for comparison (Table 5). We found no difference in 30 or 15 min from the end and end core low temperatures in relation to species in the efficacy and confirmation tests ($F = 0.77$, df = 1, $P < 0.3901$). There were differences between the confirmation and efficacy tests for the 30 or 15 min from the end core low temperatures ($F = 141.88$, df = 1, $P < 0.0001$; and $F = 43.64$, df = 1, $P < 0.001$) but not for the end core low temperature ($F = 3.49$, df = 1, $P < 0.0758$). In general, the efficacy tests resulted in lower core temperatures at the 30 or 15 min from the end than the confirmation tests (Table 5). This is primarily due to the efficacy tests being performed in the commercial CATTS chamber and the load being situated in the bottom of the lowest bin position. Core temperature was more consistent from run to run in the laboratory-scale chamber, which was most likely the result of smaller treatment loads and the chamber residing in a controlled environment.

Treatment efficacy was obtained in treatments with core low temperatures at 30 or 15 min from the end as well as end core temperatures of 39.2, 41.7, and 43.5°C, respectively. However, treatment parameters should

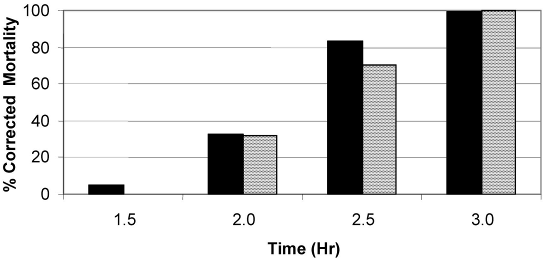

Fig. 5. Percentage of corrected mortality of fourth instars of codling moth (■) and oriental fruit moth (▤) to 12°C/h CATTS treatments (46°C, 1% O_2, 15% CO_2, 3 h). Treatments were suboptimal, in which only chamber temperature and total treatment times were met. Core temperature requirements were not adhered to.

Table 4. Confirmation test of fourth instars of codling moth by using a CATTS treatment

	No. infested	No. out	No. treated	No. live	No. dead	% mortality	Corrected no. treated
Control	1,345	324	0	1,005	16	1.5	
Treated	39,800	8,469	31,331	0	31,331	100	30,861

Treatment conditions were of 12°C/h under a 1% O_2, 15% CO_2, −2°C dew point, 1.2 m/s air speed environment to a final chamber temperature of 46°C.

Table 5. Core low temperatures of apples during the last 30 and 15 min from the end of the CATTS treatment as well as the ending core low temperature

Species	Test type	No. runs	30 min (avg °C ± SEM)	30 min (°C min.)	15 min (avg °C ± SEM)	15 min (°C min.)	End temp (avg °C ± SEM)	End temp (°C min.)
Codling moth	Confirm	20	44.6 ± 0.03a	44.3	44.9 ± 0.02a	44.6	45.0 ± 0.03a	44.7
Codling moth	Efficacy	2	40.6 ± 1.4b	39.2	42.9 ± 1.25b	41.7	44.5 ± 1.05a	43.5
Oriental fruit moth	Efficacy	2	41.2 ± 0.05b	41.2	43.3 ± 0.20b	43.1	45.0 ± 0.40a	44.6
Both[a]	Efficacy	4	40.9 ± 0.60	39.2	43.1 ± 0.52	41.7	44.8 ± 0.48	43.5
Both[a]	Both	24	44.0 ± 0.30	39.2	44.6 ± 0.15	41.7	45.0 ± 0.07	43.5

Means within a column containing the same letter are not statistically different. There were no significant differences of means in relation to species.

[a] Pooled samples not included in ANOVA analysis.

reflect those treatments in which the highest number of insects were killed, thus minimum core temperatures of 44.0, 44.6, and 45.0°C are recommended for times 30 and 15 min from the end of the treatment as well as the end core temperature (Table 5), respectively.

Discussion

It seems that the addition of a controlled atmosphere to a heat treatment either masks or blocks the acclimation process in codling moth and oriental fruit moth. Previous published research (Yokoyama and Miller 1987, Yokoyama et al. 1991) demonstrated that codling moth fourth to fifth instars are the most thermal-tolerant immature stages in this species and that they are also more thermal tolerant than the most tolerant stage of oriental fruit moth fourth instars. Further research (Neven and Mitcham 1996) indicated that the addition of a controlled atmosphere to a heat treatment would reduce the duration needed to achieve 100% mortality by nearly 50%. We later showed (Neven 2005) that it is necessary to have both elevated carbon dioxide and low oxygen to achieve optimal insect mortality of codling moth in sweet cherries (*Prunus* spp.).

The variation in mortality, which was reflected in the large confidence limits for the lethal time $(LT)_{50}$ values made it difficult to definitely determine the most tolerant stage by using Probit analysis. Factorial ANOVA indicated that there is no difference in tolerance of the larval stages, but they are more tolerant than the egg stages. It is interesting to note that the fourth instar of both species seemed to be more tolerant than the other stages and that both species were equally tolerant to the treatment. Although the fifth instar of codling moth was approximately equal to the fourth instar in tolerance, it is much more difficult to work with when attempting to infest fruit for treatment. There is a propensity for the fifth instars not to enter the fruit and form cocoons, making attainment of appropriate numbers needed for treatment difficult and expensive. Thus, the choice was made to use the fourth instar of both species to perform efficacy and confirmation tests.

Fruit Quality. We have previously published the results of fruit quality after CATTS treatments (Neven et al. 2001); nevertheless, it is appropriate to highlight the results of that research in this article. It was determined that fruit treated with heat alone (12°C/h heating rate to chamber temperatures of 44 or 46°C, −2°C dew point, 2 m/s air speed, in air) were not as firm as fruit treated with the combination heat plus CA (heating rate of 12°C/h to chamber temperatures of 44 or 46°C, −2°C dew point, 2 m/s air speed, under 1% oxygen and 15% carbon dioxide). All heat-treated fruit were firmer than untreated control fruit. Heat-treated fruit stored as long as the untreated control fruit. In Golden Delicious apples, sunburn was more prevalent in control fruit but not as common in heat treated fruit. Gala apples were more susceptible to internal breakdown after heat plus CA treatments. Red Delicious apples withstood the treatments very well. 'Fuji' apples had severe water core and did not withstand >90 d of storage. 'Granny Smith' apples stored for 150 d showed a dramatic suppression of storage scald in the heat-treated fruit. Control fruit had 100% scald, whereas heat-treated fruit were virtually untouched by storage scald. In all heat-treated fruit, the Brix/acid ratio (SS/TA) was increased, which is generally reflected in a sweeter tasting fruit.

In conclusion, the CATTS treatment effectively controls all the infestive life stages of both codling moth and oriental fruit moth. We have demonstrated that CATTS treatments can be effective in both laboratory and commercial CATTS units. We also have demonstrated from previous research (Neven et al. 2001) that CATTS treatment can result in acceptable apple quality. We think that this treatment can provide quarantine security for organic apples attempting to gain market access to countries currently requiring direct postharvest quarantine treatments while maintaining organic registration.

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References Cited

Abbott, W. S. 1925. A method of computing the effectiveness of an insecticide. J. Econ. Entomol. 18: 265–267.

Armstrong, J. W. 1994. Heat and cold treatments, pp. 103–119. *In* R. E. Paull and J. W. Armstrong [eds.], Insect pests and fresh horticultural products: treatments and responses. CAB International, Wallingford, United Kingdom.

Hallman, G. J. 1994. Controlled atmospheres, pp. 121–136. *In* R. E. Paull and J. W. Armstrong [eds.], Insect pests and fresh horticultural products: treatments and responses. CAB International, Wallingford, United Kingdom.

Klein, J. D. 1994. Time, temperature, and calcium interact in scald reduction and firmness retention in heated apples. HortScience 29: 194–195.

Klein, J. D., and S. Lurie. 1992. Prestorage heating of apple fruit for enhanced postharvest quality: interaction of time and temperature. HortScience 27: 326–328.

Klein, J. D., S. Lurie, and R. Ben-Arie. 1990. Quality and cell wall components of 'Anna' and 'Granny Smith' apples treated with heat, calcium and ethylene. J. Am. Soc. Hortic. Sci. 115: 954–958.

Lurie, S., J. D. Klein, and R. Ben Arie. 1990. Quality and cell wall components of 'Anna' and 'Granny Smith' apples treated with heat, calcium, and ethylene. J. Am. Soc. Hortic. Sci. 115: 954–958.

Lurie, S., J. D. Klein, and R. Ben-Arie. 1991. Postharvest heat treatment as a possible means of reducing superficial scald of apples. Hort. Sci. 65: 503–509.

McEvoy, M. 2006. Organic certification in the United States and Europe. (http://agr.wa.gov/FoodAnimal/Organic/docs/OrganicCertificationintheUSandEurope.pdf).

Neven, L. 1994. Combined heat treatments and cold storage effects on mortality of fifth-instar codling moth (Lepidoptera: Tortricidae). J. Econ. Entomol. 87: 1262–1265.

Neven, L. G. 1998a. Effects of heating rate on the mortality of fifth instar codling moth. J. Econ. Entomol. 91: 297–301.

Neven, L. G. 1998b. Respiratory response of fifth instar codling moth to rapidly changing temperatures. J. Econ. Entomol. 91: 302–308.

Neven, L. 2003. Effects of physical treatments on insects. HortTechnology 13: 272–275.

Neven, L. G. 2000. Insect physiological responses to heat. Postharv. Biol. Technol. 21: 103–111.

Neven, L. G. 2004. Hot forced air with controlled atmospheres for disinfestation of fresh commodities, pp. 297–315. *In* R. Dris and S. M. Janin [eds.], Production practices and quality assessment of food crops, vol. 4: post harvest treatments. Kluwer Academic Publishers, Amsterdam, The Netherlands.

Neven, L. G. 2005. Combined heat and controlled atmosphere quarantine treatments for control of codling moth, *Cydia pomonella*, in sweet cherries. J. Econ. Entomol. 98: 709–715.

Neven, L. G., and S. R. Drake. 2000a. Comparison of alternative quarantine treatments for sweet cherries. Postharv. Biol. Technol. 20: 107–114.

Neven, L. G., and S. R. Drake. 2000b. Effects of the rate of heating on apple and pear fruit quality. J. Food Qual. 23: 317–325.

Neven, L., and E. Mitcham. 1996. CATTS (controlled atmosphere/temperature treatment system): a novel tool for the development of quarantine treatments. Am. Entomol. 42: 56–59.

Neven, L., and L. Rehfield. 1995. Comparison of pre-storage heat treatments on fifth instar codling moth (Lepidoptera: Tortricidae) mortality. J. Econ. Entomol. 88: 1371–1375.

Neven, L. G., L. M. Rehfield, and K. C. Shellie. 1996. Moist and vapor forced air treatments of apples and pears: effects on the mortality of fifth instar codling moth (Lepidoptera: Tortricidae). J. Econ. Entomol. 89: 700–704.

Neven, L. G., S. R. Drake, and K. Shellie. 2001. Development of a high temperature controlled atmosphere quarantine treatment for pome and stone fruits. Acta Hortic. 553: 457–460.

[NOP] National Organic Program. 2006. The National Organic Program. (http://www.ams.usda.gov/nop/indexIE.htm).

[NWHC] Northwest Horticultural Council. 2006. Export manual. (http://www.nwhort.org/).

Obenland, D., P. Neipp, B. Mackey, and L. G. Neven. 2005. Peach and nectarine quality following treatment with high temperature forced air combined with controlled atmospheres. HortScience 40: 1425–1430.

SAS Institute. 2000. SAS user's guide: statistics. SAS Institute, Cary, NC.

Shellie, K. C., L. G. Neven, and S. R. Drake. 2001. Assessing 'Bing' sweet cherry tolerance to a heated controlled atmosphere for insect pest control. HortTechnology 11: 308–311.

Toba, H. H., and J. F. Howell. 1991. An improved system for mass-rearing codling moths. J. Entomol. Soc. Br. Columbia 88: 22–27.

Wang, S., S. L. Birla, J. Tang, and J. D. Hansen. 2006. Postharvest treatment to control codling moth in fresh apples using water assisted radio frequency heating. Postharv. Biol. Technol. 40: 89–96.

Whiting, D. C., and L. E. Hoy. 1997. High-temperature controlled atmosphere and air treatments to control obscure mealybug (Hemiptera: Pseudococcidae) on apples. J. Econ. Entomol. 90: 546–550.

Whiting, D. C., L. E. Jamieson, K. J., Spooner, and M. Lay-Yee. 1999. Combination high-temperature controlled atmosphere and cold storage as a quarantine treatment against *Ctenopseustis obliquana* and *Epiphyase postvittana* on 'Royal Gala' apples. Postharvest Biol. Technol. 16: 119–126.

Yahia, E. M. 2000a. Responses and quality of mango fruit treated with insecticidal controlled atmospheres at high temperatures. Acta Hortic. 509: 479–486.

Yahia, E. M. 2000b. The mortality of artificially infested third instar larvae of *Anastrepha ludens* and *A. obliquia* in mango fruit with insecticidal controlled atmospheres at high temperatures. Acta Hortic. 509: 833–839.

Yahia, E. M. 2000c. Mortality of eggs and third instar larvae of *Anastrepha ludens* and *A. obliqua* with insecticidal controlled atmospheres at high temperatures. Postharv. Biol. Technol. 20: 295–302.

Yocum, G., and D. L. Denlinger. 1994. Anoxia blocks thermotolerance and the induction of rapid cold hardening in the flesh fly, *Sarcophaga crassipalpis.* Physiol. Entomol. 19: 152–158.

Yokoyama, V. Y., and G. T. Miller. 1987. High temperature for control of oriental fruit moth (Lepidoptera: Tortricidae) in stone fruit. J. Econ. Entomol. 80: 641–645.

Yokoyama, V. Y., G. T. Miller, and R. V. Dowell. 1991. Response of codling moth (Lepidoptera: Tortricidae) to high temperature, a potential quarantine treatment for exported commodities. J. Econ. Entomol. 84: 528–531.

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